(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
27 March 2008 (27.03.2008)

PCT

(10) International Publication Number
WO 2008/036312 A1

(51) International Patent Classification:
A61K 31/00 (2006.01) *A61P 37/02* (2006.01)
A61K 31/4745 (2006.01) *A61P 31/10* (2006.01)

(21) International Application Number:
PCT/US2007/020296

(22) International Filing Date:
19 September 2007 (19.09.2007)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/826,151 19 September 2006 (19.09.2006) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

WO 2008/036312 A1

(54) Title: FUNGICIDAL METHODS USING IMMUNE RESPONSE MODIFIER COMPOUNDS

(57) Abstract: The present invention provides methods of killing fungal cells using immune response modifier compounds. In one aspect, the method generally includes contacting an immune response modifier (IRM) compound with a fungicidal effector cell, thereby activating the fungicidal effector cell, and allowing the activated fungicidal effector cell to kill fungal cells. In another aspect, the method generally includes contacting a fungicidal IRM compound with fungal cells in an amount effective to kill fungal cells.

FUNGICIDAL METHODS USING IMMUNE RESPONSE MODIFIER COMPOUNDS

Background

There has been a major effort in recent years, with significant success, to discover new drug compounds that act by stimulating certain key aspects of the immune system, as well as by suppressing certain other aspects (see, e.g., U.S. Pat. Nos. 6,039,969 and 6,200,592). These compounds, referred to herein as immune response modifiers (IRMs), appear to act through basic immune system mechanisms known as Toll-like receptors (TLRs) to induce selected cytokine biosynthesis, induction of co-stimulatory molecules, and increased antigen-presenting capacity.

They may be useful for treating a wide variety of diseases and conditions. For example, certain IRMs may be useful for treating viral diseases (e.g., human papilloma virus, hepatitis, herpes), neoplasias (e.g., basal cell carcinoma, squamous cell carcinoma, actinic keratosis, melanoma), and T_H2-mediated diseases (e.g., asthma, allergic rhinitis, atopic dermatitis), auto-immune diseases (e.g., multiple sclerosis), and are also useful as vaccine adjuvants. One IRM compound has been successful in one case treating a fungal infection in an immunocompetent patient. Erbagci *et al.*, *Mycopathologia* (2005), 159(4): 521-526.

Many of the IRM compounds are small organic molecule imidazoquinoline amine derivatives (see, e.g., U.S. Pat. No. 4,689,338), but a number of other compound classes are known as well (see, e.g., U.S. Pat. Nos. 5,446,153; 6,194,425; and 6,110,929; and International Publication Number WO 2005/079195) and more are still being discovered. Other IRMs have higher molecular weights, such as oligonucleotides, including CpGs (see, e.g., U.S. Pat. No. 6,194,388).

In view of the great therapeutic potential for IRMs, and despite the important work that has already been done, there is a substantial ongoing need to expand their uses and therapeutic benefits.

Summary

It has been found that certain small molecule IRMs have anti-fungal activity. As a result, these IRM molecules may be useful for killing fungal cells in therapeutic, prophylactic, diagnostic, or detection methods.

Accordingly, the present invention provides a method of killing fungal cells. Generally, the method includes contacting an immune response modifier (IRM) compound with a fungicidal effector cell, thereby activating the fungicidal effector cell, and allowing the activated fungicidal effector cell to kill fungal cells.

In another aspect, the present invention provides a method of killing fungal cells that generally includes contacting a fungicidal IRM compound with fungal cells in an amount effective to kill fungal cells.

The fungicidal activity may be exploited to treat a fungal infection in subject or, alternatively, may be the first step in a method for detecting the presence of fungal cells, generally, or cells of a particular fungal species.

Consequently, in another aspect the present invention provides a method of treating a fungal condition in a subject. Generally, the method includes administering to a subject in need of such treatment a fungicidal IRM compound in an amount effective to kill fungal cells.

In some embodiments, the fungicidal IRM compound may be administered in an amount effective to ameliorate at least one clinical sign or symptom of a fungal condition.

In other embodiments, the fungicidal IRM compound may be administered in an amount to limit or reduce the likelihood and/or extent of at least one clinical sign or symptom of a fungal condition.

In yet another aspect, the present invention provides a method of detecting fungal cells in a sample. Generally, the method includes contacting a sample with a fungicidal IRM compound, allowing fungal cells to be killed, and detecting a cell component that is detectable upon death of fungal cells.

Various other features and advantages of the present invention should become readily apparent with reference to the following detailed description, examples, claims and appended drawings. In several places throughout the specification, guidance is provided through lists of examples. In each instance, the recited list serves only as a representative group and should not be interpreted as an exclusive list.

Detailed Description of Illustrative Embodiments of the Invention

The present invention exploits the observation that many IRM compounds possess anti-fungal activity. The anti-fungal activity of the IRM compound may be due to direct anti-fungal activity, i.e., the compound itself is capable of killing or slowing the growth of fungal cells. In other cases, the anti-fungal activity may be due to indirect activity, i.e., the IRM compounds elicits an immune response that includes activating immune cells that kill or slow the growth of fungal cells. In either case, IRM compounds may be administered to a patient to treat a fungal infection, to reduce the symptoms of the fungal infection, or may be useful for performing methods that detect fungal cells and/or diagnose fungal conditions.

As used herein, the following terms shall have the indicated meanings:

"Ameliorate" refers to any reduction in the extent, severity, frequency, and/or likelihood of a symptom or clinical sign characteristic of a particular condition.

"Prophylactic" and variations thereof refer to a treatment that limits, to any extent, the development and/or appearance of a symptom or clinical sign of a condition.

"Sign" or "clinical sign" refers to an objective physical finding relating to a particular condition capable of being found by one other than the patient.

"Symptom" refers to any subjective evidence of disease or of a patient's condition.

"Therapeutic" and variations thereof refer to a treatment that ameliorates one or more existing symptoms or clinical signs associated with a condition.

"Treat" or variations thereof, when used in the context of, for example, "treating" a fungal infection, refer to reducing, limiting progression, ameliorating, or resolving, to any extent, the symptoms or signs related to a condition.

As used herein, "a," "an," "the," "at least one," and "one or more" are used interchangeably. Thus, for example, a pharmaceutical composition comprising "an" IRM compound can be interpreted to mean that the pharmaceutical composition includes at least one (i.e., one or more) IRM compound.

Also herein, the recitations of numerical ranges by endpoints include all numbers subsumed within that range (e.g., 1 to 5 includes 1, 1.5, 2, 2.75, 3, 3.80, 4, 5, etc.).

Unless otherwise indicated, reference to a compound can include the compound in any pharmaceutically acceptable form, including any isomer (e.g., diastereomer or

enantiomer), salt, solvate, polymorph, and the like. In particular, if a compound is optically active, reference to the compound can include each of the compound's enantiomers as well as racemic and scalemic mixtures of the enantiomers.

Fungicidal IRM compounds may be useful for treatment and/or diagnosis of fungal infections. Fungicidal IRM compounds may be used to provide therapeutic and/or prophylactic treatment. For example, fungicidal IRM compounds may be administered to a subject in order to reduce the likelihood or extent to which symptoms or clinical signs develop from a fungal infection. Fungicidal IRM compounds also may be administered to ameliorate at least one existing symptom or clinical sign of a fungal infection.

Alternatively, fungicidal IRM compounds may be used to detect fungal cells in a sample. For example, fungal cells may be detected in a sample by contacting the sample with a fungicidal IRM compound, followed by detection of mycospecific or species-specific cell components released and/or otherwise rendered detectable upon the death of the fungal cells.

The fungicidal activity may be direct anti-fungal activity or indirect anti-fungal activity. Direct anti-fungal activity and indirect anti-fungal activity are qualitatively distinct and rely on different mechanisms of action.

IRM compounds that possess indirect anti-fungal activity activate immune cells that have the potential, once activated, to become fungicidal effector cells. Potential fungicidal effector cells include, for example, monocytes, neutrophils, macrophages, cytotoxic T lymphocytes (CTLs), and dendritic cells. The activated effector cells possess fungicidal activity—they possess the ability to kill fungal cells. Consequently, IRM compounds that possess indirect fungicidal activity require immunocompetence—the presence of potential fungicidal effector cells. Indirect anti-fungal activity, acting through a variety of fungicidal effector cells, is demonstrated in Examples 2-5 (Tables 2-5).

IRM compounds that possess direct anti-fungal activity act directly on the fungal cells—they do not require the presence of potential fungicidal effector cells. Consequently, IRM compounds that possess direct anti-fungal activity may be useful for treating fungal infections in immuno-compromised—even immunoincompetent—subjects. Direct ant-fungal activity is demonstrated in Examples 6 and 7, below.

Fungal cells killed by practicing the present invention include, for example, Microsporum sp., Trichophyton sp., Epidermophyton sp., Aspergillus sp., Candida sp.,

Cryptococcus sp., Blastomyces sp., Histoplasma sp., Coccidioides sp., and Paracoccidioides sp.

Consequently, conditions that may be treated and/or diagnosed using an IRM compound include fungal diseases such as, for example, dermatophytosis, candidiasis, aspergillosis, histoplasmosis, blastomycosis, coccidioidomycosis, para coccidioidomycosis, and cryptococcal meningitis.

As noted above, the direct fungicidal activity of certain IRM compounds may be particularly helpful in individuals having compromised immune function. For example, certain compounds may be used for treating the fungal infections that occur after suppression of cell mediated immunity in, for example, transplant patients, cancer patients, and HIV patients.

IRM compounds include compounds that possess potent immunomodulating activity including, generally, but not limited to antiviral and antitumor activity. Certain IRMs modulate the production and secretion of cytokines. For example, certain IRM compounds induce the production and secretion of cytokines such as, e.g., Type I interferons, TNF-α, IL-1, IL-6, IL-8, IL-10, IL-12, MIP-1, and/or MCP-1. As another example, certain IRM compounds can inhibit production and secretion of certain T_H2 cytokines, such as IL-4, IL-5

Certain IRM compounds are small organic molecules (e.g., molecular weight under about 1000 Daltons, preferably under about 500 Daltons, as opposed to large biological molecules such as proteins, peptides, nucleic acids, and the like) such as those disclosed in, for example, U.S. Patent Nos. 4,689,338; 4,929,624; 5,266,575; 5,268,376; 5,346,905; 5,352,784; 5,389,640; 5,446,153; 5,482,936; 5,756,747; 6,110,929; 6,194,425; 6,331,539; 6,376,669; 6,451,810; 6,525,064; 6,541,485; 6,545,016; 6,545,017; 6,573,273; 6,656,938; 6,660,735; 6,660,747; 6,664,260; 6,664,264; 6,664,265; 6,667,312; 6,670,372; 6,677,347; 6,677,348; 6,677,349; 6,683,088; 6,756,382; 6,797,718; and 6,818,650; U.S. Patent Publication Nos. 2004/0091491; 2004/0147543; and 2004/0176367; and International Publication Nos. WO 2005/18551, WO 2005/18556, WO 2005/20999, WO 2005/032484, WO 2005/048933, WO 2005/048945, WO 2005/051317, WO 2005/051324, WO 2005/066169, WO 2005/066170, WO 2005/066172, WO 2005/076783, WO 2005/079195, WO 2006/009826, WO 2006/026760, WO 2006/028545, WO 2006/028962, WO 2006/029115, and WO 2006/065280.

Additional examples of small molecule IRM compounds include certain purine derivatives (such as those described in U.S. Patent Nos. 6,376,501, and 6,028,076), certain imidazoquinoline amide derivatives (such as those described in U.S. Patent No. 6,069,149), certain imidazopyridine derivatives (such as those described in U.S. Patent No. 6,518,265), certain benzimidazole derivatives (such as those described in U.S. Patent 6,387,938), certain derivatives of a 4-aminopyrimidine fused to a five membered nitrogen containing heterocyclic ring (such as adenine derivatives described in U. S. Patent Nos. 6,376,501; 6,028,076 and 6,329,381; and in WO 02/08905), certain 3-β-D-ribofuranosylthiazolo[4,5-d]pyrimidine derivatives (such as those described in U.S. Publication No. 2003/0199461), and certain small molecule immuno-potentiator compounds such as those described, for example, in U.S. Patent Publication No. 2005/0136065.

Other IRMs include large biological molecules such as oligonucleotide sequences. Some IRM oligonucleotide sequences contain cytosine-guanine dinucleotides (CpG) and are described, for example, in U.S. Patent Nos. 6,194,388; 6,207,646; 6,239,116; 6,339,068; and 6,406,705. Some CpG-containing oligonucleotides can include synthetic immunomodulatory structural motifs such as those described, for example, in U.S. Patent Nos. 6,426,334 and 6,476,000. Other IRM nucleotide sequences lack CpG sequences and are described, for example, in International Patent Publication No. WO 00/75304. Still other IRM nucleotide sequences include guanosine- and uridine-rich single-stranded RNA (ssRNA) such as those described, for example, in Heil *et al.*, *Science*, vol. 303, pp. 1526-1529, March 5, 2004.

Other IRMs include biological molecules such as aminoalkyl glucosaminide phosphates (AGPs) and are described, for example, in U.S. Patent Nos. 6,113,918; 6,303,347; 6,525,028; and 6,649,172.

In some embodiments of the present invention, the IRM compound may be an agonist of at least one TLR such as, for example, TLR7 or TLR8. The IRM may also in some cases be an agonist of TLR9. In some embodiments of the present invention, the IRM compound may be a small molecule immune response modifier (e.g., molecular weight of less than about 1000 Daltons).

In some embodiments of the present invention, the IRM compound may include a 2-aminopyridine fused to a five membered nitrogen-containing heterocyclic ring, or a 4-aminopyrimidine fused to a five membered nitrogen-containing heterocyclic ring.

IRM compounds suitable for use in the invention include compounds having a 2-aminopyridine fused to a five membered nitrogen-containing heterocyclic ring. Such compounds include, for example, imidazoquinoline amines including but not limited to substituted imidazoquinoline amines such as, for example, amide substituted imidazoquinoline amines, sulfonamide substituted imidazoquinoline amines, urea substituted imidazoquinoline amines, aryl ether substituted imidazoquinoline amines, heterocyclic ether substituted imidazoquinoline amines, amido ether substituted imidazoquinoline amines, sulfonamido ether substituted imidazoquinoline amines, urea substituted imidazoquinoline ethers, thioether substituted imidazoquinoline amines, hydroxylamine substituted imidazoquinoline amines, oxime substituted imidazoquinoline amines, 6-, 7-, 8-, or 9-aryl, heteroaryl, aryloxy or arylalkyleneoxy substituted imidazoquinoline amines, and imidazoquinoline diamines; tetrahydroimidazoquinoline amines including but not limited to amide substituted tetrahydroimidazoquinoline amines, sulfonamide substituted tetrahydroimidazoquinoline amines, urea substituted tetrahydroimidazoquinoline amines, aryl ether substituted tetrahydroimidazoquinoline amines, heterocyclic ether substituted tetrahydroimidazoquinoline amines, amido ether substituted tetrahydroimidazoquinoline amines, sulfonamido ether substituted tetrahydroimidazoquinoline amines, urea substituted tetrahydroimidazoquinoline ethers, thioether substituted tetrahydroimidazoquinoline amines, hydroxylamine substituted tetrahydroimidazoquinoline amines, oxime substituted tetrahydroimidazoquinoline amines, and tetrahydroimidazoquinoline diamines; imidazopyridine amines including but not limited to amide substituted imidazopyridine amines, sulfonamide substituted imidazopyridine amines, urea substituted imidazopyridine amines, aryl ether substituted imidazopyridine amines, heterocyclic ether substituted imidazopyridine amines, amido ether substituted imidazopyridine amines, sulfonamido ether substituted imidazopyridine amines, urea substituted imidazopyridine ethers, and thioether substituted imidazopyridine amines; 1,2-bridged imidazoquinoline amines; 6,7-fused cycloalkylimidazopyridine amines; imidazonaphthyridine amines; tetrahydroimidazonaphthyridine amines; oxazoloquinoline amines; thiazoloquinoline amines; oxazolopyridine amines;

thiazolopyridine amines; oxazolonaphthyridine amines; thiazolonaphthyridine amines; pyrazolopyridine amines; pyrazoloquinoline amines; tetrahydropyrazoloquinoline amines; pyrazolonaphthyridine amines; tetrahydropyrazolonaphthyridine amines; and 1*H*-imidazo dimers fused to pyridine amines, quinoline amines, tetrahydroquinoline amines, naphthyridine amines, or tetrahydronaphthyridine amines.

In certain embodiments, the IRM compound may be an imidazonaphthyridine amine, a tetrahydroimidazonaphthyridine amine, an oxazoloquinoline amine, a thiazoloquinoline amine, an oxazolopyridine amine, a thiazolopyridine amine, an oxazolonaphthyridine amine, a thiazolonaphthyridine amine, a pyrazolopyridine amine, a pyrazoloquinoline amine, a tetrahydropyrazoloquinoline amine, a pyrazolonaphthyridine amine, or a tetrahydropyrazolonaphthyridine amine.

In certain embodiments, the IRM compound may be a substituted imidazoquinoline amine, a tetrahydroimidazoquinoline amine, an imidazopyridine amine, a 1,2-bridged imidazoquinoline amine, a 6,7-fused cycloalkylimidazopyridine amine, an imidazonaphthyridine amine, a tetrahydroimidazonaphthyridine amine, an oxazoloquinoline amine, a thiazoloquinoline amine, an oxazolopyridine amine, a thiazolopyridine amine, an oxazolonaphthyridine amine, a thiazolonaphthyridine amine, a pyrazolopyridine amine, a pyrazoloquinoline amine, a tetrahydropyrazoloquinoline amine, a pyrazolonaphthyridine amine, or a tetrahydropyrazolonaphthyridine amine.

As used herein, a substituted imidazoquinoline amine refers to an amide substituted imidazoquinoline amine, a sulfonamide substituted imidazoquinoline amine, a urea substituted imidazoquinoline amine, an aryl ether substituted imidazoquinoline amine, a heterocyclic ether substituted imidazoquinoline amine, an amido ether substituted imidazoquinoline amine, a sulfonamido ether substituted imidazoquinoline amine, a urea substituted imidazoquinoline ether, a thioether substituted imidazoquinoline amine, a hydroxylamine substituted imidazoquinoline amine, an oxime substituted imidazoquinoline amine, a 6-, 7-, 8-, or 9-aryl, heteroaryl, aryloxy or arylalkyleneoxy substituted imidazoquinoline amine, or an imidazoquinoline diamine. As used herein, substituted imidazoquinoline amines specifically and expressly exclude 1-(2-methylpropyl)-1*H*-imidazo[4,5-*c*]quinolin-4-amine and 4-amino-α,α-dimethyl-2-ethoxymethyl-1*H*-imidazo[4,5-*c*]quinolin-1-ethanol.

In one embodiment, an IRM compound having direct fungicidal activity may be an imidazoquinoline amine, a tetrahydroimidazoquinoline amine, an imidazopyridine amine, a 1,2-bridged imidazoquinoline amine, a 6,7-fused cycloalkylimidazopyridine amine, an imidazonaphthyridine amine, a tetrahydroimidazonaphthyridine amine, an oxazoloquinoline amine, a thiazoloquinoline amine, an oxazolopyridine amine, a thiazolopyridine amine, an oxazolonaphthyridine amine, a thiazolonaphthyridine amine, a pyrazolopyridine amine, a pyrazoloquinoline amine, a tetrahydropyrazoloquinoline amine, a pyrazolonaphthyridine amine, or a tetrahydropyrazolonaphthyridine amine. An IRM compound with direct fungicidal activity may be, for example, a sulfonamide substituted imidazoquinoline amine such as, for example, N-[4-(4-amino-2-ethyl-1*H*-imidazo[4,5-*c*]quinolin-1-yl)butyl]methanesulfonamide

In another embodiment, an IRM compound having indirect fungicidal activity may be a substituted imidazoquinoline amine, a tetrahydroimidazoquinoline amine, an imidazopyridine amine, a 1,2-bridged imidazoquinoline amine, a 6,7-fused cycloalkylimidazopyridine amine, an imidazonaphthyridine amine, a tetrahydroimidazonaphthyridine amine, an oxazoloquinoline amine, a thiazoloquinoline amine, an oxazolopyridine amine, a thiazolopyridine amine, an oxazolonaphthyridine amine, a thiazolonaphthyridine amine, a pyrazolopyridine amine, a pyrazoloquinoline amine, a tetrahydropyrazoloquinoline amine, a pyrazolonaphthyridine amine, or a tetrahydropyrazolonaphthyridine amine. An IRM compound having indirect fungicidal activity may be, for example, a tetrahydroimidazoquinoline amine such as, for example, 4-amino-2-(ethoxymethyl)-α,α-dimethyl-6,7,8,9-tetrahydro-1*H*-imidazo[4,5-*c*]quinoline-1-ethanol.

Suitable IRM compounds also may include the purine derivatives, imidazoquinoline amide derivatives, benzimidazole derivatives, adenine derivatives, aminoalkyl glucosaminide phosphates, small molecule immuno-potentiator compounds, and oligonucleotide sequences described above. In some embodiments, the IRM compound may be a compound identified as an agonist of one or more TLRs.

Examples of particular IRM compounds include, but are not limited to N-(2-(4-amino-2-(ethoxymethyl)-1H-imidazo[4,5-c]quinolin-1-yl)-1,1-dimethylethyl)methanesulfonamide, 5-(4-amino-2-methyl-1H-imidazo[4,5-c]quinolin-1-yl)-4,4-dimethylpentan-2-one, N-(4-(4-amino-2-methyl-6,7,8,9-tetrahydro-1H-imidazo[4,5-c]quinolin-1-yl)butyl)morpholine-4-carboxamide, and 1-(4-amino-2-ethyl-2H-pyrazolo[3,4-c]quinolin-1-yl)-2-methylpropan-2-ol.

For *in vitro* applications such as diagnostic or detection assays, the IRM compound may be provided in any suitable formulation. The IRM compound may be provided in any suitable form including but not limited to a solution, a suspension, and emulsion, or any

form of mixture. The formulation may include additional active components such as, for example, other anti-fungal components, antibodies, markers, buffers, and the like.

For *in vivo* applications, the compound may be provided in any formulation suitable for administration to a subject. Suitable types of formulations are described, for example, in U.S. Pat. No. 5,238,944; U.S. Pat. No. 5,939,090; U.S. Pat. No. 6,245,776; European Patent No. EP 0 394 026; and U.S. Patent Publication No. 2003/0199538. The compound may be provided in any suitable form including but not limited to a solution, a suspension, an emulsion, or any form of mixture. The compound may be delivered in formulation with any pharmaceutically acceptable excipient, carrier, or vehicle. For example, the formulation may be delivered in a conventional topical dosage form such as, for example, a cream, an ointment, an aerosol formulation, a non-aerosol spray, a gel, a lotion, and the like. The formulation may further include one or more additives including but not limited to adjuvants, skin penetration enhancers, colorants, fragrances, flavorings, moisturizers, thickeners, and the like.

An *in vivo* formulation may be administered in any suitable manner such as, for example, non-parenterally or parenterally. As used herein, non-parenterally refers to administration through the digestive tract, including by oral ingestion. Parenterally refers to administration other than through the digestive tract such as, for example, intravenously, intramuscularly, transdermally, subcutaneously, transmucosally (e.g., by inhalation), or topically.

The composition of an *in vivo* formulation suitable for practicing the invention will vary according to factors known in the art including but not limited to the physical and chemical nature of the IRM compound, the nature of the carrier, the intended dosing regimen, the state of the subject's immune system (e.g., suppressed, compromised, stimulated), the method of administering the IRM compound, and the species to which the formulation is being administered. Accordingly, it is not practical to set forth generally the composition of a formulation effective for all possible applications. Those of ordinary skill in the art, however, can readily determine an appropriate formulation with due consideration of such factors.

In some embodiments, the methods of the present invention include administering IRM to a subject in a formulation of, for example, from about 0.0001% to about 20% (unless otherwise indicated, all percentages provided herein are weight/weight with

respect to the total formulation) to the subject, although in some embodiments the IRM compound may be administered using a formulation that provides IRM compound in a concentration outside of this range. In certain embodiments, the method includes administering to a subject a formulation that includes from about 0.01% to about 1% IRM compound, for example, a formulation that includes about from about 0.1 % to about 0.5% IRM compound.

An amount of an IRM compound effective for killing fungal cells is an amount sufficient to either directly kill fungal cells or induce fungicidal effector cells to kill fungal cells. The precise amount of IRM compound that is effective for killing fungal cells will vary according to factors known in the art including but not limited to the physical and chemical nature of the IRM compound, the nature of the carrier, the intended dosing regimen, the state of the subject's immune system (e.g., suppressed, compromised, stimulated), the method of administering the IRM compound, and the species to which the formulation is being administered. Accordingly, it is not practical to set forth generally the amount that constitutes an amount of IRM compound effective for all possible applications. Those of ordinary skill in the art, however, can readily determine the appropriate amount with due consideration of such factors.

In some embodiments, the methods of the present invention include administering sufficient IRM compound to provide a dose of, for example, from about 100 ng/kg to about 50 mg/kg to the subject, although in some embodiments the methods may be performed by administering IRM compound in a dose outside this range. In some of these embodiments, the method includes administering sufficient IRM compound to provide a dose of from about 10 μg/kg to about 5 mg/kg to the subject, for example, a dose of from about 100 μg/kg to about 1 mg/kg.

Alternatively, the dose may be calculated using actual body weight obtained just prior to the beginning of a treatment course. For the dosages calculated in this way, body surface area (m^2) is calculated prior to the beginning of the treatment course using the Dubois method: $m^2 = (\text{wt kg}^{0.425} \times \text{height cm}^{0.725}) \times 0.007184$.

In some embodiments, the methods of the present invention may include administering sufficient IRM compound to provide a dose of, for example, from about 0.01 mg/m^2 to about 10 mg/m^2.

The dosing regimen may depend at least in part on many factors known in the art including but not limited to the physical and chemical nature of the IRM compound, the nature of the carrier, the amount of IRM being administered, the state of the subject's immune system (e.g., suppressed, compromised, stimulated), the method of administering the IRM compound, and the species to which the formulation is being administered. Accordingly it is not practical to set forth generally the dosing regimen effective for all possible applications. Those of ordinary skill in the art, however, can readily determine an appropriate dosing regimen with due consideration of such factors.

In some embodiments of the invention, the IRM compound may be administered, for example, from a single dose to multiple doses per day. In certain embodiments, the IRM compound may be administered on an "as needed" basis, i.e., as frequently as needed to ameliorate a clinical sign or symptom of a fungal infection. In certain embodiments, the IRM compound may be administered from about once per day to once per month such as, for example, once per day.

The methods of the present invention may be performed on any suitable subject. Suitable subjects include but are not limited to animals such as but not limited to humans, non-human primates, poultry, fowl, rodents, dogs, cats, horses, pigs, sheep, goats, or cows.

Examples

The following examples have been selected merely to further illustrate features, advantages, and other details of the invention. It is to be expressly understood, however, that while the examples serve this purpose, the particular materials and amounts used as well as other conditions and details are not to be construed in a matter that would unduly limit the scope of this invention.

IRM Compounds

The IRM compounds used in the examples are shown in Table 1.

Table 1

Compound	Chemical Name	Reference
IRM1	4-amino-2-(ethoxymethyl)-α,α-dimethyl-6,7,8,9-tetrahydro-1*H*-imidazo[4,5-*c*]quinoline-1-ethanol	U.S. 5,352,784 Example 91

Compound	Chemical Name	Reference
IRM2	N-[4-(4-amino-2-ethyl-1*H*-imidazo[4,5-*c*]quinolin-1-yl)butyl]methanesulfonamide	U.S. 6,677,349 Example 236

Example 1

Blood was obtained from female BALB/c mice (Charles Rivers Lab, Willmington, MA) by cardiac puncture into EDTA vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ). Blood was pooled; diluted 1:1 (vol:vol) with Phosphate Buffered Saline (PBS) or Hank's Balance Salt Solution (HBSS), diluted blood was overlayed on ACCUPAQUE (Accurate Chemical and Scientific Co., Westbury, NY) 1:1 (vol:vol), and centrifuged (30 minutes, 200 x g, room temperature (RT)). The mononuclear cell interface of the centrifuged mixture was removed, washed with PBS or HBSS, centrifuged (7 minutes, 60 x g, RT), and resuspended in 10% RPMI complete media to a final cell concentration of 4 x 10^6 per mL. 10% RPMI complete media consisted of RPMI 1640 (Celox Laboratories, Inc., St. Paul, MN); 1% (vol:vol) each of HEPES (Cat. No. P305-100, Biofluids, Inc., Rockville, MD), MEM non-essential amino acid solution (Cat. No. M7145, Sigma Chemical Co., St. Louis, MO), L-glutamine (Cat. No. G7513, Sigma Chemical Co.), penicillin-streptomycin (Cat. No. P0781, Sigma Chemical Co.), sodium pyruvate (Cat. No. P333-100, Biofluids, Inc.); and 0.1% (vol:vol) 2-mercaptoethanol (Cat. No. 21985-023, Gibco, Grand Island, NY). Cells were treated with 1 or 3 μM of IRM1, DMSO, or untreated at a final cell concentration of 2 x 10^6 per mL in a 48-well tissue culture plate (Corning, Inc., Corning, NY) and incubated overnight at 37°C in 5% CO_2. Plates were centrifuged (10 minutes, 200 x g, 4°C), supernatants were removed and frozen for future use.

Example 2

Blood was obtained from 7 to 8 week old male BALB/c mice (Simonsen Laboratories, Inc., Gilroy, CA) by axillary bleeding and heparinzed (10 U/mL). Blood was mixed 1:1 (vol:vol) in saline, 4 mL of the mixture was layered over 4 mL of HISTOPAQUE 1077 (Sigma Chemical Co., St. Louis, MO) and centrifuged for 30 minutes at 400 x g. The peripheral blood mononuclear cells (PBMCs) were collected,

diluted with RPMI-1640 (Sigma Chemical Co.) and pelleted by centrifugation (10 minutes, 400 x g). PBMCs were resuspended in RPMI media containing 10% fetal bovine serum (FBS), 100 U/mL penicillin, 100 µg/mL streptomycin at a concentration of 5×10^6 cells/mL in this complete tissue culture media (CTCM). The PBMCs were dispensed at 200 µL per well (Costar Cat. No. 5936, Corning, Inc., Corning, NY), incubated for two hours at 37°C in 5% CO_2, and non-adherent cells were removed by aspiration. The number of adherent monocytes per well was estimated to be 5×10^5 per well by subtracting the number of non-adherent cells from the number of total cells originally plated.

The monocytes were then treated with 200 µL per well of CTCM alone or containing DMSO; CTCM containing 250 or 1000 U/mL of recombinant mouse IFN-γ (Genentech, Inc., South San Francisco, CA); or the supernatants from Example 1. Monocytes were incubated for 20 hours at 37°C in 5% CO_2, supernatants were aspirated, and monocytes were challenged with 200 µL per well of CTCM containing *Candia albicans* at a monocyte to *C. albicans* ratio of 50:1 for two hours at 37°C in 5% CO_2. Cultures were harvested by aspiration into distilled water and plate wells were washed 10 times with distilled water. The harvested material was plated on BAP medium in duplicate, incubated at 35°C for 20 hours, colony counts were made, and colony-forming units (CFUs) per culture well calculated.

Fungicidal activity was defined as a reduction of inoculum's CFU and calculated using the following formula to determine the percent killing: [1-(inoculum's CFU/experimental CFU) X 100]. Statistics were performed between the groups using the Student's t-test with significance at $P < 0.05$. The GB-Stat program (Dynamic Microsystems, Inc., Silver Spring, MD) was used and Bonferroni's adjustment to the t-test was used when appropriate. Results are shown in Table 2 below.

Table 2

	CFU/well	Percentage of Killing (%)	P value	P value
T0	1900±60		Ref	
T2	1720±160	10	NS	
DMSO	2100±40	0	NS	
Control	1960±120	0	NS	Ref
IRM1 [1 µM]	1780±160	7	NS	NS

IRM1 [3 μM]	1520±220	20	<0.05	<0.05
IFN-γ [1000 U/mL]	1600±20	16	<0.01	<0.05
IFN-γ [250 U/mL]	1480±100	23	<0.05	<0.05

NS=Not significant P>0.05

Example 3

Blood was obtained from mice as described in Example 2. Pelleted PMBCs, prepared as described in Example 2, were collected in saline and mixed 1:1 (vol:vol) in 3% Dextran 500 (wt:vol saline) and allowed to sediment for one hour at 1 x g, 37°C. The white blood cell layer (PMN) was collected and centrifuged (10 minutes, 400 x g). Pelleted cells were treated with 0.85% NH_4Cl to lyse contaminating red blood cells. PMNs were suspended in CTCM, CTCM containing DMSO, CTCM containing recombinant mouse IFN-γ, or the supernatants from Example 1 and plated at 1 x 10^5 PMNs/well. PMNs were incubated, supernatants were aspirated, challenged with *C. albicans*, harvested, and CFU counts were made as described in Example 2. Fungicidal activity was determined and statistics were performed as described in Example 2. Results are shown in Table 3 below.

Table 3

	CFU/well	Percentage of Killing (%)	P value	P value
T0		0		
T2	2340±160	0	Ref	
DMSO	1420±100	40	<0.01	
Control	1380±160	42	<0.01	Ref
IRM1 [1 μM]	640±60	73	<0.01	<0.01
IRM1 [3 μM]	800±160	66	<0.01	<0.01
IFN-γ [1000 U/mL]	300±60	88	<0.01	<0.01
IFN-γ [500 U/mL]	640±100	73	<0.01	<0.01
IFN-γ [250 U/mL]	760±40	68	<0.01	<0.01

NS=Not significant P>0.05

Example 4

Peritoneal cells (PC) were collected by lavage using 10 mL of RPMI in the peritoneal cavities of male BALB/c mice (10 mice per experiment). PC were pelleted by centrifugation (10 minutes at 400 x g), resuspended in CTCM at a concentration of 2 x 10^6/mL, 200 μL of cells were plated per well, incubated for two hours at 37°C in 5% CO_2,

and non-adherent cells were removed by aspiration. The number of adherent cells (peritoneal macrophages, PMs) per well was estimated to be 1 x 10^5 per well by subtracting the number of non-adherent cells from the number of total cells originally plated. PMs were treated with CTCM, CTCM containing DMSO, CTCM containing recombinant mouse IFN-γ, or the supernatants from Example 1. PMs were incubated, supernatants were aspirated, challenged with *C. albicans*, harvested, and CFU counts were made as described in Example 2. Fungicidal activity was determined and statistics were performed as described in Example 2. Results are shown in Table 4 below.

Table 4

	CFU/well	Percentage of Killing (%)	P value	P value
T0	1900±40	0	Ref	
T2	1720±160	10	NS	
DMSO	1860±80	3	NS	
Control	1900±80	0	NS	Ref
IRM1 [1 μM]	1480±140	23	<0.01	<0.01
IRM1 [3 μM]	1780±20	7	NS	NS
IFN-γ [1000 U/mL]	1200±60	37	<0.01	<0.01

NS=Not significant P>0.05

Example 5

Peritoneal macrophages were isolated as described in Example 4. The PMs were treated with 0.1, 1.0, or 10 μg/mL of IRM1; 1000 U/mL recombinant mouse IFN-γ, or CTCM alone. Cells were incubated for 20 hours at 37°C in 5% CO_2, supernatants were aspirated and PMs were challenged with *C. albicans* at a 100:1 ratio for two hours at 37°C in 5% CO_2. Harvested materials from the plate and CFU counts were made as described in Example 2. Fungicidal activity was determined and statistics were performed as described in Example 2. Results are shown in Table 5 below.

Table 5

	CFU/well	Percentage of Killing (%)	P value
T0	2020±80	0	
T2	1760±60	12	
Macrophages alone	1760±220	12	Ref

IRM1 [0.1 μg/mL]	1200±80	40	<0.01
IRM1 [1 μg/mL]	1260±40	37	<0.05
IRM1 [10 μg/mL]	1100±40	45	<0.01
IFN-γ [1000 U/mL]	1440±200	28	NS

NS=Not significant P>0.05

Example 6

IRM2 was dissolved in a 0.0005 M citric acid solution (pH 3.65) or 0.0125 M acetic acid solution (pH 3.61) by adding 100 milligrams of IRM2 to 5 mL of either solution. The IRM2 solutions were added to wells containing 100 μL of either Fluid Sabaroud's Medium (FSM) or a defined media of 30% sucrose/0.01% peptone and 5 μL of inoculum containing approximately 1 x 10^4 colony forming units (CFUs) of *Aspergillus niger* (ATCC No. 16404, Manassas, VA). Triplicate serial two-fold dilutions of the IRM2 solution from 100 μL to 3.1 μL or no IRM were added to each well and limulus amoebocyte lysate (LAL) reagent water, if needed, was added to each well to a final well volume of 205 μL. Plates were incubated at 35°C for 7 days. Growth of *A. niger* was determined visually by comparing turbidity to the control well containing no IRM2. No hyphal elements were present in the 100 μL IRM2 citric acid solution well. No hyphal elements were present in the 100 μL, 50 μL, and 25 μL IRM2 acetic acid solution wells.

Example 7

IRM2 was tested for antifungal activity as described in Example 6. The amount of the IRM2 solution added to each well were 100 μL, 75 μL, 50 μL, 25 μL, or 12.5 μL. No hyphal elements were present in the 100 μL or 75 μL IRM2 citric acid solution wells. No hyphal elements were present in the 100 μL, 75 μL, 50 μL, and 25 μL IRM2 acetic acid solution wells.

The complete disclosures of the patents, patent documents and publications cited herein are incorporated by reference in their entirety as if each were individually incorporated. In case of conflict, the present specification, including definitions, shall control.

Various modifications and alterations to this invention will become apparent to those skilled in the art without departing from the scope and spirit of this invention.

Illustrative embodiments and examples are provided as examples only and are not intended to limit the scope of the present invention. The scope of the invention is limited only by the claims set forth as follows.

What is Claimed is:

1. A method of killing fungal cells, the method comprising:

contacting an immune response modifier (IRM) compound with a fungicidal effector cell, thereby activating the fungicidal effector cell; and

allowing the activated fungicidal effector cell to kill fungal cells, wherein the IRM compound comprises a substituted imidazoquinoline amine, a tetrahydroimidazoquinoline amine, an imidazopyridine amine, a 1,2-bridged imidazoquinoline amine, a 6,7-fused cycloalkylimidazopyridine amine, an imidazonaphthyridine amine, a tetrahydroimidazonaphthyridine amine, an oxazoloquinoline amine, a thiazoloquinoline amine, an oxazolopyridine amine, a thiazolopyridine amine, an oxazolonaphthyridine amine, a thiazolonaphthyridine amine, a pyrazolopyridine amine, a pyrazoloquinoline amine, a tetrahydropyrazoloquinoline amine, a pyrazolonaphthyridine amine, or a tetrahydropyrazolonaphthyridine amine.

2. The method of claim 1 wherein the fungicidal effector cell comprises a monocyte.

3. The method of claim 1 wherein the fungicidal effector cell comprises a neutrophil.

4. The method of claim 1 wherein the fungicidal effector cell comprises a macrophage.

5. The method of claim 1 wherein the fungicidal effector cell comprises a cytotoxic T lymphocyte.

6. The method of claim 1 wherein the fungicidal effector cell comprises a dendritic cell.

7. The method of claim 1 wherein the IRM compound comprises a tetrahydroimidazoquinoline amine.

8. The method of claim 1 wherein the IRM compound contacts the fungicidal effector cell *in vivo*.

9. The method of claim 1 wherein the IRM compound contacts the fungicidal effector cell *ex vivo*.

10. A method of killing fungal cells, the method comprising:

contacting a fungicidal IRM compound with fungal cells in an amount effective to kill fungal cells, wherein the IRM compound comprises an imidazoquinoline amine, a tetrahydroimidazoquinoline amine, an imidazopyridine amine, a 1,2-bridged imidazoquinoline amine, a 6,7-fused cycloalkylimidazopyridine amine, an imidazonaphthyridine amine, a tetrahydroimidazonaphthyridine amine, an oxazoloquinoline amine, a thiazoloquinoline amine, an oxazolopyridine amine, a thiazolopyridine amine, an oxazolonaphthyridine amine, a thiazolonaphthyridine amine, a pyrazolopyridine amine, a pyrazoloquinoline amine, a tetrahydropyrazoloquinoline amine, a pyrazolonaphthyridine amine, or a tetrahydropyrazolonaphthyridine amine.

11. The method of claim 10 wherein the fungicidal IRM compound comprises a substituted imidazoquinoline amine.

12. The method of claim 11 wherein the fungicidal IRM compound comprises a sulfonamide substituted imidazoquinoline amine.

13. The method of claim 10 wherein the IRM contacts the fungal cells *in vivo*.

14. The method of claim 13 wherein the IRM contacts the fungal cells in an immunoincompetent subject.

15. The method of claim 10 wherein the IRM compound contacts the fungal cells *ex vivo*.

16. A method of detecting fungal cells in a sample, the method comprising:
contacting a sample with a fungicidal IRM compound;
allowing fungal cells to be killed;
and detecting a cell component that is detectable upon death of fungal cells.

17. The method of claim 16 wherein the fungicidal IRM compound possesses direct anti-fungal activity.

18. The method of claim 16 wherein the fungicidal IRM compound contacts at least one fungicidal effector cell, activates the fungicidal effector cell, and the activated fungicidal effector cell kills at least one fungal cell.

19. A method of treating a fungal infection, the method comprising:
administering tom a subject in need of such treatment a fungicidal IRM compound in an amount effective to kill fungal cells.

20. The method of claim 19 wherein the fungicidal IRM compound is administered in an amount to ameliorate at least one symptom or clinical sign of the fungal infection.

21. The method of any one of claims 1-20 wherein the fungal cell comprises Microsporum sp.

22. The method of any one of claims 1-20 wherein the fungal cell comprises Trichophyton sp.

23. The method of any one of claims 1-20 wherein the fungal cell comprises Epidermophyton sp.

24. The method of any one of claims 1-20 wherein the fungal cell comprises Aspergillus sp.

25. The method of any one of claims 1-20 wherein the fungal cell comprises Candida sp.

26. The method of any one of claims 1-20 wherein the fungal cell comprises Cryptococcus sp.

27. The method of any one of claims 1-20 wherein the fungal cell comprises Blastomyces sp.

28. The method of any one of claims 1-20 wherein the fungal cell comprises Histoplasma sp.

29. The method of any one of claims 1-20 wherein the fungal cell comprises Coccidioides sp.

30. The method of any one of claims 1-20 wherein the fungal cell comprises Paracoccidioides sp.

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2007/020296

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/00 A61K31/4745 A61P37/02 A61P31/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 03/043572 A (3M INNOVATIVE PROPERTIES CO [US]) 30 May 2003 (2003-05-30) page 15, line 4 - line 5 page 16, line 19 - line 24 page 18, line 4 - line 9	1-30

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 January 2008	08/02/2008

Name and mailing address of the ISA/	Authorized officer
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FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box II.1

Although claims 1-30 are directed to a diagnostic method practised on the human/animal body, the search has been carried out and based on the alleged effects of the compound.

Continuation of Box II.1

Claims Nos.: -

Rule 39.1(iv) PCT - Method for treatment of the human or animal body by therapy

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2007/020296

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: –
because they relate to subject matter not required to be searched by this Authority, namely:

see FURTHER INFORMATION sheet PCT/ISA/210

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers allsearchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International search reportcovers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

[ ] The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2007/020296

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 03043572 A	30-05-2003	AU 2002343728 A1	10-06-2003
		EP 1455700 A2	15-09-2004
		JP 2005513021 T	12-05-2005
		JP 2006249102 A	21-09-2006